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THE THIN LAYER CHROMATOGRAPHY OF ALKYL AND HALOGEN SUBSTITUTED BENZALDEHYDE 2,4-DINITROPHENYLHYDRAZONES

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THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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TO MY WIFE, CAROL

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ABSTRACT

The thin layer chromatographic separation of alkyl and halogen substituted benzaldehyde 2,4-dinitrophenylhydrazones has been studied using a zinc carbonate adsorbant on glass plates and a 90% carbon disulfide-10% chloroform solvent mixture as the elutant. The halogen substituted benzaldehyde 2,4-dinitrophenylhydrazones were separated from the standard compound, benzaldehyde 2,4-dinitrophenylhydrazone. Of the alkyl derivatives examined only 4-isopropyl benzaldehyde 2,4-dinitrophenylhydrazone separated.

The adsorptive properties of thin layers of zinc carbonate and alumina were investigated and an eluotropic series for each adsorbant was established.

INTRODUCTION

A convenient method of characterizing carbonyl compounds has been to form the 2,4-dinitrophenylhydrazone (2,4-DNPH). Since these compounds are intensely colored, scientists have studied the application of chromatographic technique to the separation and isolation of 2,4-DNPH derivatives of carbonyl compounds. Paper[1-3], thin layer[4-22], column[23], and vapor phase[24] chromatography have been used. It is the purpose of this experimental work to study the thin layer chromatographic separation of alkyl and halogen substituted benzaldehyde 2,4-DNPH's relative to benzaldehyde 2,4-DNPH.

One of the earliest studies of the thin layer chromatography of aromatic carbonyl compounds was that of Dhont and De Reoy[6]. In 1965 Ruffini[14] reported the R_f values of 2,4-DNPH's of 32 aromatic aldehydes and ketones. These compounds were chromatographed on silica gel G, using a 2:1 mixture of ethyl acetate and ligroine as elutant. The separation of as many as four derivatives from a mixture was achieved. In these early studies no alkyl or halogen substituted benzaldehyde 2,4-DNPH's were investigated.

Finley and Gilman[16] reported on the thin layer chromatography of substituted benzaldehyde 2,4-DNPH's. Ten of the nineteen substituted 2,4-DNPH's that were chromatographed as components of binary mixtures were found to be separable. This data is presented in Table I. It may be noted that the alkyl and halogen substituted benzaldehyde 2,4-DNPH's were not separable.

TABLE I

$R_{benzaldehyde}$ Values of Substituted Benzaldehyde 2,4-DNPH's

Substituent	I*	2	3
4-OH	0.07	0.07	0.09
3-OH	0.09	0.10	0.11
4-CN	0.36	0.37	0.30
$4-NO_2$	0.51	0.53	0.54
$3-NO_2$	0.64	0.63	0.63
$3-OCH_3$	0.73	0.82	0.76
$4-OCH_3$	0.73	0.78	0.72
$4-N(CH_3)_2$	0.73	0.75	0.72
2-OH	0.78	0.82	0.70
$4-N(C_2H_5)_2$	0.93	0.91	0.87
4-F	0.96	0.98	0.93
4-Cl	0.98	0.97	0.96
4-Br	0.98	1.04	1.11
$4-CH(CH_3)_2$	0.98	1.04	1.06
3-Cl	1.00	1.01	1.00
3-Br	1.00	0.99	1.06
3-F	1.02	1.01	0.96
$3-CH_3$	1.07	1.00	1.04
$4-CH_3$	1.07	1.03	1.00

* Apparatus and conditions: (I) tank, benzene, Chromagram sheet, silica gel; (2) Chromagram sandwich, benzene, Chromagram sheet, silica gel; (3) tank, benzene, glass plate, silica gel G.

Jart and Bigler[18] studied the migration of the 2,4-DNPH's of carbonyl compounds on microplates (40 X 75mm.) of alumina. The chromatographs were developed using a solvent mixture consisting of 15 ml. of ethyl acetate-100 ml. of petroleum ether (62-82°). The $R_{benzaldehyde}$ values of some pertinent compounds are found in Table II.

TABLE II

$R_{benzaldehyde}$ Values of Substituted Benzaldehyde 2,4-DNPH's

Substituent	$R_{benzaldehyde}$
4-Cl	0.99
4-Br	0.99
4-CH_3	1.03

Apparatus and conditions: 250 ml. beaker, ethyl acetate-petroleum ether, microplates, alumina.

Clearly the alkyl and halogen substituted benzaldehyde 2,4-DNPH's investigated did not separate relative to the standard compound in the experimental work presented above. Badings and Wassink[9] in 1963 reported the chromatographic separation of mixtures of the 2,4-DNPH's of aliphatic aldehydes derived from the autoxidation of lipids. Chromatoplates of zinc carbonate were developed in a solvent mixture of petroleum ether-benzene-pyridine (7:1:2) employing thin layer, and then partition chromatography. Using these techniques Badings was able to separate compounds of

strikingly similar polarity. Although the compounds I was investigating fell into a different class, a study of the adsorptive properties of thin layers of zinc carbonate was undertaken. It was hoped that a thin layer chromatographic system would be found that would provide a means of separating alkyl and halogen substituted benzaldehyde 2,4-DNPH's relative to benzaldehyde 2,4-DNPH.

RESULTS AND DISCUSSION

Separation of Binary Mixtures

The thin layer chromatographic separation of alkyl and halogen substituted benzaldehyde 2,4-DNPH's relative to the standard compound, benzaldehyde 2,4-DNPH, is reported in Table III. The values presented are defined relative to the standard compound[25] in the following manner:

$$R_{benzaldehyde} = \frac{R_f \text{ substituted benzaldehyde 2,4-DNPH}}{R_f \text{ benzaldehyde 2,4-DNPH}}$$

TABLE III

$R_{benzaldehyde}$ Values of Substituted Benzaldehyde 2,4-DNPH's

Substituent	$R_{benzaldehyde}$
4-F	0.74
4-Br	0.78
4-Cl	0.79
3-Cl	0.84
3-Br	0.86
3-F	0.87
$3\text{-}CH_3$	1.00
$4\text{-}CH_3$	1.00
$4\text{-}CH(CH_3)_2$	1.10

Apparatus and conditions: tank, 90% carbon disulfide - 10% chloroform, glass plate, zinc carbonate.

Seven of the nine substituted benzaldehyde 2,4-DNPH's that were chromatographed as binary mixtures were separable from the standard compound benzaldehyde 2,4-DNPH. This may be attributed to the polar nature of the substituent in the case of the halogen substituted compounds. All of these compounds were found to migrate slower than the standard.

The methyl substituted benzaldehyde 2,4-DNPH's show R_f values close to that of benzaldehyde 2,4-DNPH and when chromatographed as mixtures were inseparable from the standard. In a study that was carried out simultaneously, Freytag and Ney[20] reported the separation of 3 and 4-methyl benzaldehyde 2,4-DNPH's from benzaldehyde 2,4-DNPH on chromatoplates of kieselgel G. These were developed in a solvent mixture of petroleum ether (40-60°)-chloroform-ethyl acetate (30:3:1). The $R_{benzaldehyde}$ values for these compounds are presented in Table IV. It should be noted

TABLE IV

$R_{benzaldehyde}$ Values of Substituted Benzaldehyde 2,4-DNPH's

Substituent	$R_{benzaldehyde}$
3-CH_3	1.10
4-CH_3	1.09

Apparatus and conditions: tank, petroleum ether (40-60°)-chloroform-ethyl acetate (30:3:1), glass plate, kieselgel G.

that these values are for each compound chromatographed separately, and not as the component of a binary mixture with benzaldehyde 2,4-DNPH.

4-Isopropyl benzaldehyde 2,4-DNPH was found to be separable, migrating faster than benzaldehyde 2,4-DNPH. This is due to the aliphatic nature of the isopropyl group, which increases the solubility of this compound in the elutant. The high $R_{benzaldehyde}$ value is also due to the lower affinity of the adsorbant for the compound, which again results from the presence of the isopropyl group.

The isomeric halogen substituted compounds were found to be separable under the conditions of our experiment. The para substituted compounds migrated significantly slower than the meta substituted compounds. This may be attributed to the electron donating resonance effect of the halogen substituent in the para position.

In Figure I a representative chromatoplate is shown.

Precision of Experimental Work

The precision of this experimental work is expressed in terms of the standard deviation of 9-12 $R_{benzaldehyde}$ values for each of the compounds studied. These are presented in Table V. These standard deviations lie within the limits of $\pm$ 0.05 which is generally considered acceptable. The precision of a larger number of determinations was obtained by taking the standard deviation of some 50 R_f values of benzaldehyde 2,4-DNPH. It was found to be $\pm$ 0.326, which although less precise, lies within the limits normally found satisfactory.

TABLE V

Precision of $R_{benzaldehyde}$ Values of Substituted Benzaldehyde 2,4-DNPH's

Substituent	
4-F	± 0.019
4-Br	± 0.020
4-Cl	± 0.020
3-Cl	± 0.012
3-Br	± 0.015
3-F	± 0.015
3-CH_3	± 0.009
4-CH_3	± 0.008
4-$CH(CH_3)_2$	± 0.022

Eluotropic Series of Solvents[26]

An eluotropic series, a series of solvents in order of increasing eluting power, was established for the adsorbants, zinc carbonate and alumina. This information was obtained from the data collected in selecting the thin layer chromatographic system used in our experimentation. The eluotropic series for the adsorbant zinc carbonate appears in Table V, and that for alumina in Table VI. The eluting power of the solvent is reported in terms of the R_f value of the standard compound, benzaldehyde 2,4-DNPH. The larger the R_f value, the greater the eluting power of the solvent.

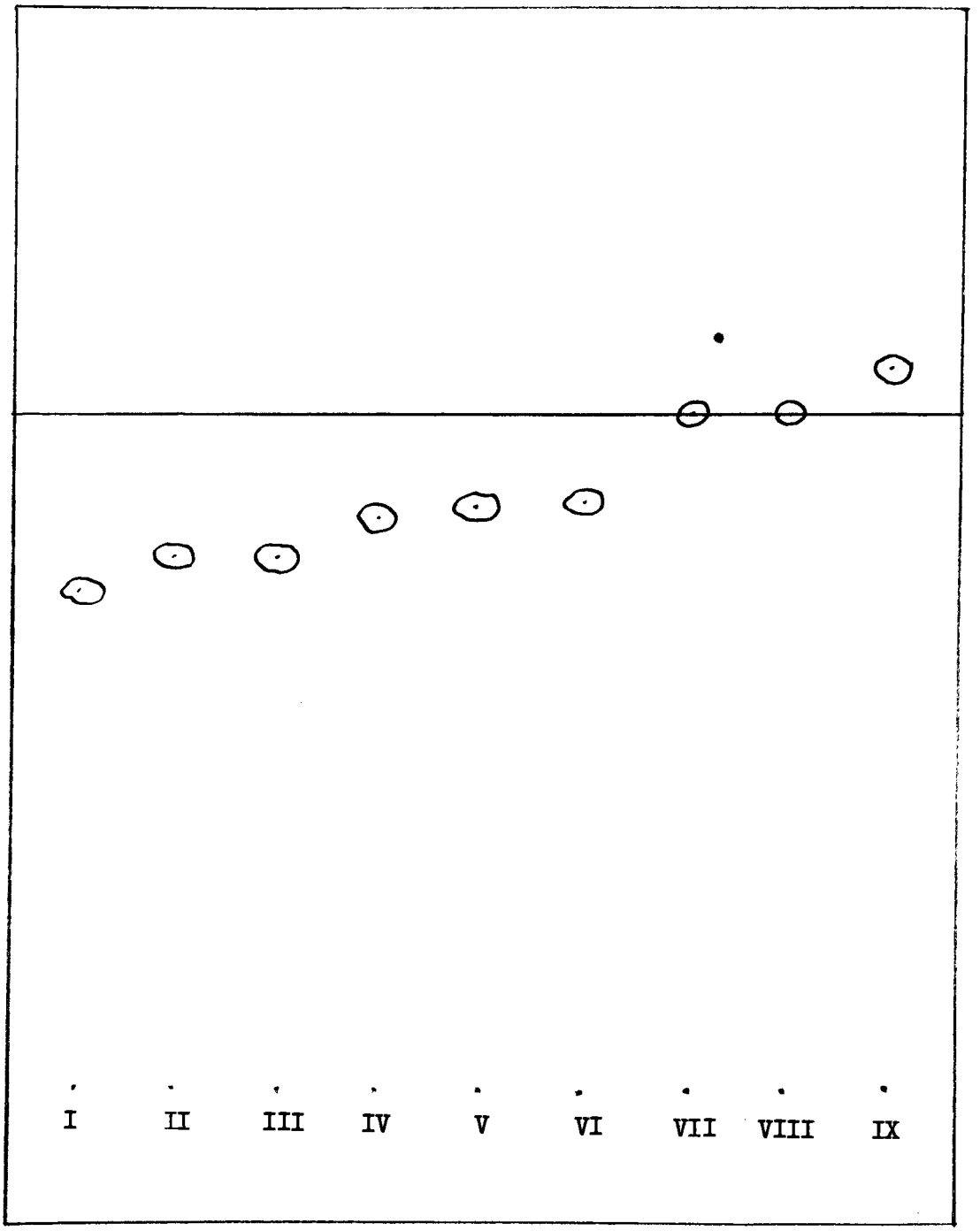

Figure I Migration of Substituted Benzaldehyde 2,4-DNPH's Substituents - I-4-F, II-4-Br, III-4-Cl, IV-3-Cl, V-3-Br, VI-3-F, VII-3-CH_3, VIII-4-CH_3, IX-4-$CH(CH_3)_2$.

TABLE VI

Eluotropic Series of Solvents-Zinc Carbonate

Elutant	R_f
85% carbon disulfide-15% acetone	0.92
50% acetone-50% cyclohexane	0.87
tetrahydrafuran	0.87
acetone	0.84
dioxane	0.82
80% benzene-20% ethyl acetate	0.77
benzene	0.76
90% carbon disulfide-10% chloroform	0.66
75% toluene-25% cyclohexane	0.56
60% benzene-40% cyclohexane	0.48
carbon tetrachloride	0.24
95% carbon disulfide-5% benzene	0.15
cyclohexane	0.05
carbon disulfide	0.00

Apparatus and conditions: tank, glass plate, zinc carbonate.

TABLE VII

Eluotropic Series of Solvents-Alumina

Elutant	R_f
90% benzene-10% methanol	0.69
50% benzene-50% chloroform	0.68
dioxane	0.68
ethyl acetate	0.60
85% carbon sulfide-15% acetone	0.57
80% chloroform-20% cyclohexane	0.49
benzene	0.39
90% carbon disulfide-10% chloroform	0.26
60% benzene-40% cyclohexane	0.23
carbon disulfide	0.08

Apparatus and conditions: tank, Eastman chromagram sheet, alumina.

EXPERIMENTAL

The Preparation of 2,4-Dinitrophenylhydrazones[27]

A solution of 2,4-dinitrophenylhydrazine was prepared in the following manner. To 0.8 g. of 2,4-dinitrophenylhydrazine in a 125 ml. erlenmeyer flask was added 2 ml. of concentrated sulfuric acid. Water (6 ml.) was added dropwise, with swirling and stirring until solution was complete. To this warm solution was added 20 ml. of 95% ethanol.

A solution of the carbonyl compound in ethanol was prepared by dissolving 1.0 g. of the compound in 20 ml. of 95% ethanol. The freshly prepared 2,4-dinitrophenylhydrazine solution was added, and the resulting mixture was allowed to stand at room temperature. Crystallization of the 2,4-dinitrophenylhydrazone usually occurred within five to ten minutes. If no precipitate was formed, the mixture was allowed to stand overnight.

The 2,4-DNPH's were recrystallized in 95% ethanol until a constant melting point was reached. Corrected melting points, obtained using a Meltemp apparatus, are found in Table VII.

Standardized Thin Layer Chromatographic Procedure

Preparation of the Chromatographic Plates The glass plates were readied for spreading in the following manner. The plates were washed with a solution of alconox, and thoroughly rinsed with distilled water. These were then hand dried with clean towels and placed on a standard Desaga mounting board[28]. The slurry was prepared by adding 70 ml. of distilled water to 25 g. of the

TABLE VIII

Corrected Melting Points of 2,4-DNPH's

Carbonyl Compound	Melting Point	
	Literature[29,30,31]	Observed
Benzaldehyde	237	236-237
3-Methylbenzaldehyde	212	211-212
4-Methylbenzaldehyde	232-234	231-234
4-Isopropylbenzaldehyde	241-244	241
3-Fluorobenzaldehyde	268	267-268
4-Fluorobenzaldehyde	278	275-278
3-Chlorobenzaldehyde	248	246-248
4-Chlorobenzaldehyde	254	252-255
3-Bromobenzaldehyde	256-257	257
4-Bromobenzaldehyde	257	255-257

adsorbant, reagent grade zinc carbonate with a 5% starch binder. An analysis of the zinc carbonate used is found in Appendix I. This was stirred continuously until a homogeneous mixture was formed. Care was taken to avoid formation of lumps and bubbles in the slurry. It was immediately transferred to a Desaga adjustable applicator[32] (model S-11-S) and the plates were spread. The thickness of the chromatographic layer was 250 microns. The plates were then placed on a drying rack and allowed to air dry for 24 hours.

Activation of the Chromatographic Plates The plates were activated in a drying oven at a temperature of 110 for one hour, and stored in a stainless steel desiccating cabinet until used.

Application of the Sample The spotting technique employed in the application of the sample was that of Finley and Gilman[16]. The solutions for spotting were prepared by dissolving 10 mg. of compound in 100 ml. of chloroform. Spots of 3-5 (6-10 λ for mixtures) were applied so that their diameter did not exceed 4 mm. The spots were located 20 mm. from the bottom of the glass plate. The distance from the edge and between adjacent spots was also 20 mm. After the spots had air dried for 15 minutes, the plate was ready to be developed.

Three spots of a given binary mixture (benzaldehyde and a substituted benzaldehyde 2,4-DNPH) and three spots of each component of the mixture were chromatographed on a large glass plate (20 X 20 cm.). Figure 2 shows a representative chromatogram.

Development of Chromatographic Plates The standard Desaga developing tanks were readied in the following fashion. Each tank was lined with Whatman #1 filter paper. The elutant, a mixture of 90% carbon disulfide-10% chloroform by volume, was added until the solvent depth in the tank was 15 mm. The tank was then sealed and set aside for one hour, allowing the upper portions of the chamber to become saturated with the vapors of the elutant. At this point the chromatographic plate was placed in the tank and it was resealed. During development the eluting solvent traveled a distance of 150 mm. from the point of application of the sample. The plate was then removed and the R_f values were recorded.

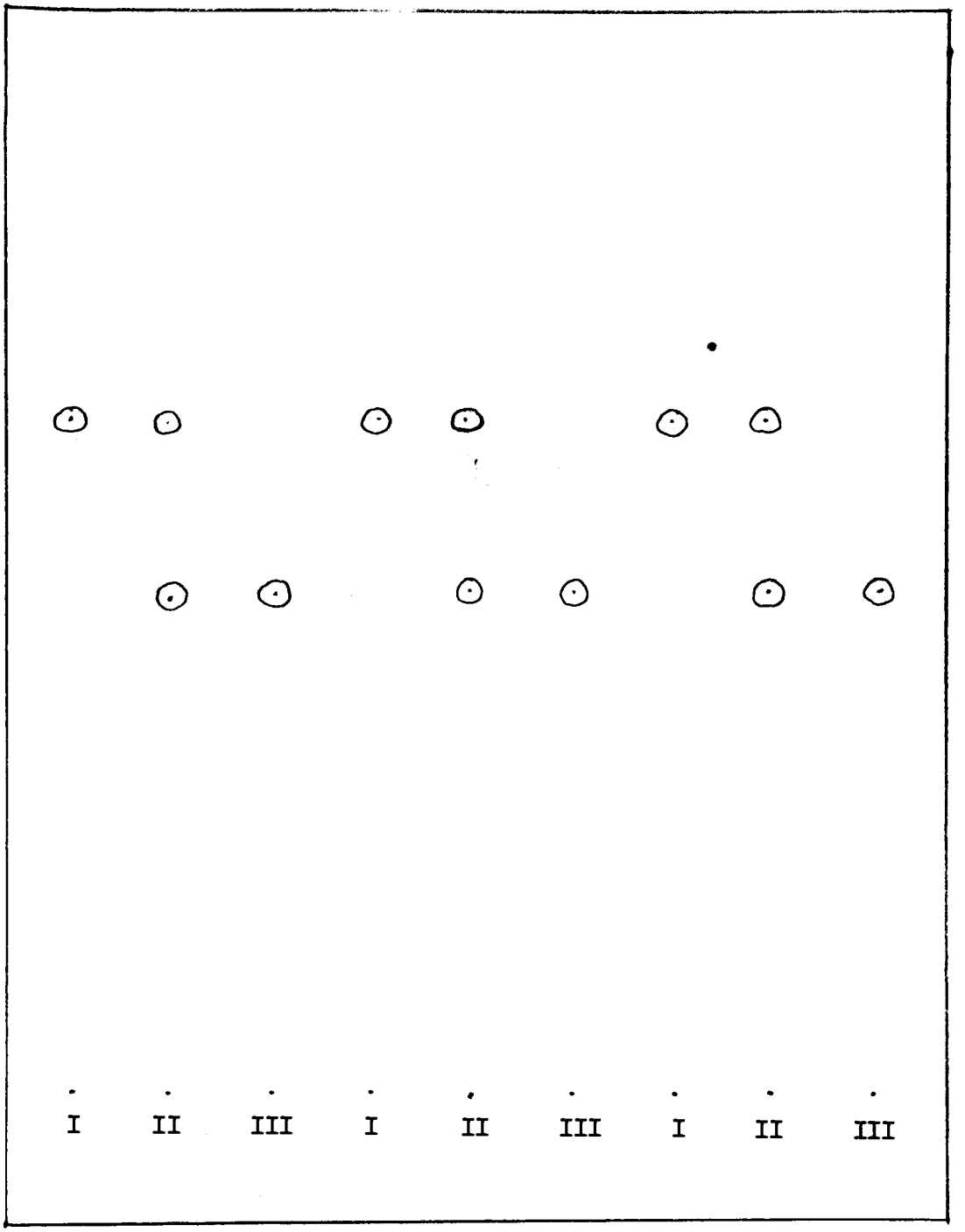

Figure II Separation of Binary Mixtures of 2,4-DNPH's I-Benzaldehyde 2,4-DNPH, II-binary mixture, III-4-Fluorobenzaldehyde 2,4-DNPH.

APPENDIX I

Analysis of Zinc Carbonate *

Assay (as ZnO)	71.5	%
Insoluble in H_2SO_4	0.005	%
Chloride (Cl)	0.001	%
Nitrate (NO_3)	0.001	%
Sulfate (SO_4)	0.003	%
Lead (Pb)	0.003	%
Iron (Fe)	0.0005	%
Substances not Precipitated by $(NH_4)_2S$ (as SO_4)	0.40	%

* J. T. Baker Laboratory Chemicals Co., Cat. No. 4312, Lot No. 21251

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